SHOCK, Vol. 41, Supplement 1, pp. 51–53, 2014

Review Article

REFRIGERATED PLATELETS FOR THE TREATMENT OF ACUTE BLEEDING: A REVIEW OF THE LITERATURE AND REEXAMINATION OF CURRENT STANDARDS

Heather F. Pidcoke,* Philip C. Spinella,† Anand K. Ramasubramanian,‡ Geir Strandenes,§‖ Tor Hervig,‖ Paul M. Ness,¶ and Andrew P. Cap*

**Blood Research Program, US Army Institute of Surgical Research, Fort Sam Houston, Texas; †Department of Critical Care, Department of Pediatrics, Washington University in St Louis, Missouri; ‡Department of Biomedical Engineering, The University of Texas at San Antonio, San Antonio, Texas; §Norwegian Naval Special Operation Commando and ‖Department of Immunology and Transfusion Medicine, Haukeland University Hospital, Bergen, Norway; and ¶Division of Transfusion Medicine, Department of Pathology, Johns Hopkins Hospital, Baltimore, Maryland*

Received 17 Sep 2013; first review completed 3 Oct 2013; accepted in final form 18 Oct 2013

ABSTRACT—This review is a synopsis of the decisions that shaped global policy on platelet (PLT) storage temperature and a focused appraisal of the literature on which those discussions were based. We hypothesize that choices were centered on optimization of preventive PLT transfusion strategies, possibly to the detriment of the therapeutic needs of acutely bleeding patients. Refrigerated PLTs are a better hemostatic product, and they are safer in that they are less prone to bacterial contamination. They were abandoned during the 1970s because of the belief that clinically effective PLTs should both be hemostatically functional and survive in circulation for several days as indicated for prophylactic transfusion; however, clinical practice may be changing. Data from two randomized controlled trials bring into question the concept that stable autologous stem cell transplant patients with hypoproliferative thrombocytopenia should continue to receive prophylactic transfusions. At the same time, new findings regarding the efficacy of cold PLTs and their potential role in treating acute bleeding have revived the debate regarding optimal PLT storage temperature. In summary, a "one-size-fits-all" strategy for PLT storage may not be adequate, and a reexamination of whether cold-stored PLTs should be offered as a widely available therapeutic product may be indicated.

KEYWORDS—Cold platelets, severe hemorrhage, acute hemorrhage, hypoproliferative thrombocytopenia, trauma, platelet storage temperature, damage control resuscitation, massive transfusion protocols

ABBREVIATIONS—PLT — platelet; 4C-PLTs — refrigerated (4°C)-stored platelets; RT — room temperature; RT-PLTs — room temperature (22°C)–stored platelets; CFR — Code of Federal Regulations sections; RCT — randomized controlled trial

COLD VERSUS ROOM TEMPERATURE STORAGE OF PLATELETS FOR THE TREATMENT OF ACUTE BLEEDING

Platelet (PLT) transfusion is associated with improved clinical outcomes in acutely bleeding trauma patients (1–9). Despite common beliefs to the contrary, PLTs are available in whole-blood products; they can also be prepared as whole blood–derived concentrates, as buffy coat preparations, or as apheresis units. Currently, unlike red cell products that are stored under refrigeration, PLT units are stored at room temperature (RT) (20°C–24°C) with constant agitation for 5 days (7 days in some European countries) to maximize recovery and survival *in vivo* following transfusion, because refrigerated PLTs are cleared more rapidly from circulation (10). Platelets participate in immunomodulation, maintenance and repair of vessel structures, and, their best-known function, clot formation. Prevention of bleeding requires longer circulation times (survival), whereas hemorrhage control requires initiation of the clot (activation), which removes the PLT from circulation. Increasing PLT circulating time reduces the frequency of prophylactic PLT transfusion and thus the risk of alloimmunization in patients with hypoproliferative thrombocytopenia, a population that is primarily made up of patients receiving chemotherapy. Blood providers adopted RT-stored PLTs (RT-PLTs) because most civilian PLTs are used by patients with hypoproliferative thrombocytopenia; split inventories pose logistical problems for transfusion services, and providers believe that RT storage meets the needs of patients with acute postsurgical or traumatic bleeding. Furthermore, focusing on PLT use in cancer therapy, regulators adopted PLT *in vivo* circulation time as the primary metric of PLT function, instead of other parameters such as adhesion, aggregation, or contribution to clot strength. Unfortunately, the switch to RT-PLTs came at the cost of increased risk of bacterial contamination and decreased hemostatic function compared with cold storage (0°C–6°C) (11–16). Refrigerated PLTs (4C-PLTs) have been reported to reduce blood loss due to acute hemorrhage, are less conducive to bacterial growth, and were the standard of care until the mid-1980s.

Address reprint requests to Andrew P. Cap, MD, PhD, FACP, Blood Research Program, US Army Institute of Surgical Research, Fort Sam Houston, TX 78234. E-mail: andrew.p.cap.mil@mail.mil.

The authors declare that they have no conflicts of interest relevant to this article.

The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

DOI: 10.1097/SHK.0000000000000078

Copyright © 2014 by the Shock Society

Copyright © 2014 by the Shock Society. Unauthorized reproduction of this article is prohibited.

Report Documentation Page

Form Approved
OMB No. 0704-0188

Public reporting burden for the collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to a penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
01 MAY 2014	**N/A**	**-**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Refrigerated Platelets for the Treatment of Acute Bleeding: A Review of the Literature and Reexamination of Current Standards.	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
Pidcoke H. F., Spinella P. C., Ramasubramanian A. K., Strandenes G., Hervig T., Ness P. M., Cap A. P.,	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **US Army Institute of Surgical Research, JBSA Fort Sam Houston, Texas**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release, distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			
unclassified	**unclassified**	**unclassified**	**UU**	**3**	

The narrow focus on PLT *in vivo* circulation eclipsed other aspects of PLT function and resulted in the abandonment of 4C-PLT in the effort to reduce transfusion frequency for prophylactic transfusions (17).

This decision was not without controversy. The merits of maintaining two PLT inventories, one with longer circulating PLTs maintained at RT for prophylactic use and another with more hemostatic refrigerated PLTs for treatment of acute hemorrhage, were vigorously debated (18–20). Ultimately, RT-PLTs became the norm because hypoproliferative thrombocytopenia outnumbered other indications for PLT transfusion (21). In the intervening three decades, however, clinical practice has changed. While prophylactic transfusion continues to predominate, some centers are now reporting greater percentages of other therapeutic indications, possibly due to the lowering of the transfusion threshold in the 1990s combined with increased use by other services due to increased use of PLT inhibitors and the adoption of massive transfusion protocols to treat acutely bleeding patients (22–24). Treatment of acute hemorrhage may become the predominant reason for PLT utilization if findings from two recent large randomized controlled trials (RCTs) of prophylactic PLT transfusions result in new practice guidelines (25). Although not all experts agree, those studies indicate that hematologically stable patients who have undergone autologous stem cell transplantation can be safely managed without prophylactic transfusion (26–29). Some of the controversy is centered on the question of whether the risks in this population are limited to World Health Organization grade 2 bleeding versus more life-threatening hemorrhage (26, 28). Possible benefits of reducing prophylactic transfusion would include decreased risk of bacterial contamination, decreased donor exposure, and lower PLT utilization.

Given the prevalence of bacterial contamination, there is no question that risk decreases with fewer PLT transfusions (30–33). Conversely, donor exposure did not decrease in the therapeutic arm in one trial because there was a concomitant increase in packed red blood cell transfusion (27). The poorer hemostatic function of RT-PLTs may have contributed; they have well-recognized defects in aggregation response and, at a minimum, may require 1 to 2 h after *in vivo* administration to recover full function (15, 18). Their refrigerated counterparts, in contrast, are mildly activated and may be primed to immediately participate in clot formation (15, 34). Room temperature–stored PLT *in vivo* functional recovery has not been definitively established.

Data from two RCTs indicate that when a PLT-containing blood product is stored at 4°C, it becomes more hemostatically active than those stored at 22°C (35–36). One trial, performed in 150 children, demonstrated significantly reduced postoperative bleeding after cardiac surgery in those who received refrigerated PLT-containing whole blood, compared with those who received reconstituted whole blood containing RT PLTs (35). Differences between the refrigerated whole-blood and fresh whole-blood recipients were not significant, whereas PLT function, as measured with light impedance aggregometry, was lowest in samples from patients who received reconstituted blood with RT-PLTs (35). Furthermore, a trial in adults taking aspirin demonstrated that 4C-PLTs had significantly improved bleeding times in contrast to those at RT, which had little or delayed effects (36). Findings from these RCTs were supported by data from the 1970s demonstrating that refrigerated PLTs corrected bleeding time more quickly than did RT-PLTs (15). The foundation of damage control resuscitation and massive transfusion protocols is that early correction of coagulopathy limits blood loss, decreasing blood product transfusion (37–39). Application of this concept by adding a cold PLT arm for the patients treated with a therapeutic strategy might have checked hemorrhage progression via early administration of a more effective hemostatic product. This is supported by work in animal models of hemorrhagic shock: rodents demonstrate coagulopathy and PLT loss after a severe bleed, suggesting that transfusion of immediately viable, partially primed PLTs could be beneficial to this population (40, 41). Furthermore, military personnel wounded during the conflicts in Iraq and Afghanistan demonstrated benefit with early PLT transfusion (1, 2).

Data from the two RCTs of therapeutic PLT transfusions versus prophylactic may also have implications for the treatment of patients with severe hemorrhage due to other etiologies. Results demonstrated that the therapeutic strategy led to acceptable outcomes in most patients with hypoproliferative thrombocytopenia, with the exception of the highest-risk patients receiving induction chemotherapy or allogeneic stem cell transplantation for leukemia (25, 29). Trauma patients do not have hypoproliferative thrombocytopenia, but rather acute insufficiency due to consumption and loss. Given that these patients are able to generate their own PLT supply, which is presumably superior to any transfused product, a treatment strategy aimed at immediate hemorrhage control, rather than long-term survival of transfused PLTs, may result in fewer transfusions and better outcomes. Clinical trials to address this question are indicated, particularly because 4C-PLTs were the standard of care for over a decade, have an established safety record, and are a licensable product in the United States according to the Food and Drug Administration Code of Federal Regulations sections (CFR) 21CFR640.24 and 21CFR640.25.

Even if 4C-PLTs are merely shown to be equivalent to RT-PLTs, the logistical exigencies of far-forward care in austere environments argue for a reexamination of current standards and a more practical approach to PLT storage. Similarly, availability of an extended storage cold PLT product could greatly benefit civilian centers. As mentioned above, PLTs stored under refrigeration, whether as whole blood or PLT products, should improve hemostatic function, clinical safety, and efficacy (12–14, 35, 36, 42). Achieving hemostasis within the first 6 h following massive traumatic hemorrhage is associated with improved outcomes (3, 43). Refrigerated PLTs are cleared over 2 to 3 days; thus, they certainly remain in circulation long enough to contribute to hemostasis during the crucial initial period after injury (10). Furthermore, recent data suggest that 4C-PLTs retain hemostatic function for at least 10 days when refrigerated (12, 14). In the context of remote damage control resuscitation, whole blood or PLT units could be collected at support bases or forward locations and transported to remote sites in isothermal transport boxes (e.g., Golden Hour boxes) along with other blood products and medications. This would reduce the logistical complexity of transporting products at different temperatures, increase inventory,

Copyright © 2014 by the Shock Society. Unauthorized reproduction of this article is prohibited.

reduce risks of bacterial contamination, and facilitate early delivery of critically needed hemostatic products to bleeding trauma patients. For remote damage control resuscitation purposes, the current standard of PLT storage at RT—and by extension, limitation on whole-blood use—is impractical and wasteful and potentially deprives trauma patients of lifesaving products.

We are concerned that RT storage of PLTs may also compromise the acute care of patients in civilian medicine undergoing trauma resuscitation or surgical bleeding due to thrombocytopenia. Clinical studies of 4°C-stored PLTs or other formulations designed to have more immediate hemostatic function should be undertaken. If the data from these investigations are supportive, PLT storage protocols could be modified to provide improved hemostatic support for all patients. Whether RT storage should be maintained for patients with hypoproliferative thrombocytopenia or whether blood centers and transfusion services can manage split PLT inventories for these different hemostatic needs could then be considered based on the evolving clinical evidence. A "one-size-fits-all" strategy for PLT storage may not be adequate.

REFERENCES

1. Pidcoke HF, Aden JK, Mora AG, Borgman MA, Spinella PC, Dubick MA, Blackbourne LH, Cap AP: Ten-year analysis of transfusion in Operation Iraqi Freedom and Operation Enduring Freedom: increased plasma and platelet use correlates with improved survival. *J Trauma Acute Care Surg* 73(6 Suppl 5): S445–S452, 2012.
2. Perkins JG, Cap AP, Spinella PC, Blackbourne LH, Grathwohl KW, Repine TB, Ketchum L, Waterman P, Lee RE, Beekley AC, et al.: An evaluation of the impact of apheresis platelets used in the setting of massively transfused trauma patients. *J Trauma* 66(Suppl 4):S77–S84; discussion S84–S85, 2009.
3. Holcomb JB, Wade CE, Michalek JE, Chisholm GB, Zarzabal LA, Schreiber MA, Gonzalez EA, Pomper GJ, Perkins JG, Spinella PC, et al.: Increased plasma and platelet to red blood cell ratios improves outcome in 466 massively transfused civilian trauma patients. *Ann Surg* 248(3):447–458, 2008.
4. Perkins JG, Cap AP, Spinella PC, Shorr AF, Beekley AC, Grathwohl KW, Rentas FJ, Wade CE, Holcomb JB: Comparison of platelet transfusion as fresh whole blood versus apheresis platelets for massively transfused combat trauma patients (CME). *Transfusion* 51(2):242–252, 2011.
5. Nessen SC, Eastridge BJ, Cronk D, Craig RM, Berseus O, Ellison R, Remick K, Seery J, Shah A, Spinella PC: Fresh whole blood use by forward surgical teams in Afghanistan is associated with improved survival compared to component therapy without platelets. *Transfusion* 53(Suppl 1):107S–113S, 2013.
6. Spinella PC, Dunne J, Beilman GJ, O'Connell RJ, Borgman MA, Cap AP, Rentas F: Constant challenges and evolution of US military transfusion medicine and blood operations in combat. *Transfusion* 52(5):1146–1153, 2012.
7. Rock G, Sherring VA, Tittley P: Five-day storage of platelet concentrates. *Transfusion* 24(2):147–152, 1984.
8. Hoshi R, Murata S, Matsuo R, Myronovych A, Hashimoto I, Ikeda H, Ohkohchi N: Freeze-dried platelets promote hepatocyte proliferation in mice. *Cryobiology* 55(3):255–260, 2007.
9. Rock G, Moltzan C, Alharbi A, Giulivi A, Palmer D, Bormanis J: Automated collection of blood components: their storage and transfusion. *Transfus Med* 13(4):219–225, 2003.
10. Murphy S, Gardner FH: Effect of storage temperature on maintenance of platelet viability—deleterious effect of refrigerated storage. *N Engl J Med* 280(20):1094–1098, 1969.
11. Palavecino EL, Yomtovian RA, Jacobs MR: Bacterial contamination of platelets. *Transfus Apher Sci* 42(1):71–82, 2010.
12. Pidcoke HF, McFaul SJ, Ramasubramanian AK, Parida BK, Mora AG, Fedyk CG, Valdez-Delgado KK, Montgomery RK, Reddoch KM, Rodriguez AC, et al: Primary hemostatic capacity of whole blood: a comprehensive analysis of pathogen reduction and refrigeration effects over time. *Transfusion* 53(Suppl 1):137S–149S, 2013.
13. Montgomery RK, Reddoch KM, Evani SJ, Cap AP, Ramasubramanian AK: Enhanced shear-induced platelet aggregation due to low temperature storage. *Transfusion* 53(7):1520–1530, 2013.
14. Jobes D, Wolfe Y, O'Neill D, Calder J, Jones L, Sesok-Pizzini D, Zheng XL: Toward a definition of "fresh" whole blood: an *in vitro* characterization of coagulation properties in refrigerated whole blood for transfusion. *Transfusion* 51(1):43–51, 2011.
15. Filip DJ, Aster RH: Relative hemostatic effectiveness of human platelets stored at 4-degrees-C and 22-degrees-C. *J Lab Clin Med* 91(4):618–624, 1978.
16. Juffermans NP, Prins DJ, Vlaar AP, Nieuwland R, Binnekade JM: Transfusion-related risk of secondary bacterial infections in sepsis patients: a retrospective cohort study. *Shock* 35(4):355–359, 2011.
17. Kilkson H, Holme S, Murphy S: Platelet metabolism during storage of platelet concentrates at 22-degrees-C. *Blood* 64(2):406–414, 1984.
18. Holme S, Vaidja K, Murphy S: Platelet storage at 22-degrees-C—effect of type of agitation on morphology, viability, and function *in vitro*. *Blood* 52(2):425–435, 1978.
19. Kattlove HE: Platelet preservation—what temperature—rationale for strategy. *Transfusion* 14(4):328–330, 1974.
20. Valeri CR: Circulation and hemostatic effectiveness of platelets stored at 4 C or 22 C: studies in aspirin-treated normal volunteers. *Transfusion* 16(1):20–23, 1976.
21. Slichter SJ: Controversies in platelet transfusion therapy. *Annu Rev Med* 31:509–540, 1980.
22. Greeno E., McCullough J, Weisdorf D: Platelet utilization and the transfusion trigger: a prospective analysis. *Transfusion* 47(2):201–205, 2007.
23. Lin YC, Chang CS, Yeh CJ, Wu YC: The appropriateness and physician compliance of platelet usage by a computerized transfusion decision support system in a medical center. *Transfusion* 50(12):2565–2570, 2010.
24. Qureshi H, Lowe D, Dobson P, Grant-Casey J, Parris E, Dalton D, Hickling K, Waller F, Howell C, Murphy MF: National comparative audit of the use of platelet transfusions in the UK. *Transfus Clin Biol* 14(6):509–513, 2007.
25. Wandt H, Schaefer-Eckart K, Wendelin K, Pilz B, Wilhelm M, Thalheimer M, Mahlknecht U, Ho A, Schaich M, Kramer M, et al.: Therapeutic platelet transfusion versus routine prophylactic transfusion in patients with haematological malignancies: an open-label, multicentre, randomised study. *Lancet* 380(9850): 1309–1316, 2012.
26. Wandt H, Schafer-Eckart K, Ehninger G: Prophylactic platelet transfusion. *N Engl J Med* 369(6):577, 2013.
27. Slichter SJ: Prophylactic platelet transfusion. *N Engl J Med* 369(6): 577–578, 2013.
28. Slichter SJ: Eliminate prophylactic platelet transfusions? *N Engl J Med* 368(19):1837–1838, 2013.
29. Stanworth SJ, Estcourt LJ, Powter G, Kahan BC, Dyer C, Choo L, Bakrania L, Llewelyn C, Littlewood T, Soutar R, et al.: A no-prophylaxis platelet-transfusion strategy for hematologic cancers. *N Engl J Med* 368(19):1771–1780, 2013.
30. Mathai J: Problem of bacterial contamination in platelet concentrates. *Transfus Apher Sci* 41(2):139–144, 2009.
31. Palavecino E, Jacobs M, Yomtovian R: Bacterial contamination of blood products. *Curr Hematol Rep* 3(6):450–455, 2004.
32. Ness P, Braine H, King K, Barrasso C, Kickler T, Fuller A, Blades N: Single-donor platelets reduce the risk of septic platelet transfusion reactions. *Transfusion* 41(7):857–861, 2001.
33. Braine HG, Kickler TS, Charache P, Ness PM, Davis J, Reichart C, Fuller AK: Bacterial sepsis secondary to platelet transfusion: an adverse effect of extended storage at room temperature. *Transfusion* 26(4):391–393, 1986.
34. Hoffmeister KM, Felbinger TW, Falet H, Denis CV, Bergmeier W, Mayadas TN, von Andrian UH, Wagner DD, Stossel TP, Hartwig JH: The clearance mechanism of chilled blood platelets. *Cell* 112(1):87–97, 2003.
35. Manno CS, Hedberg KW, Kim HC, Bunin GR, Nicolson S, Jobes D, Schwartz E, Norwood WI: Comparison of the hemostatic effects of fresh whole blood, stored whole blood, and components after open heart surgery in children. *Blood* 77(5):930–936, 1991.
36. Becker GA, Tuccelli M, Kunicki T, Chalos MK, Aster RH: Studies of platelet concentrates stored at 22 C and 4 C. *Transfusion* 13(2):61–68, 1973.
37. Tourtier JP, Palmier B, Tazarourte K, Raux M, Meaudre E, Ausset S, Sailliol A, Vivien B, Domanski L, Carli P: The concept of damage control: extending the paradigm in the prehospital setting. *Ann Fr Anesth Reanim* 32(7–8):520–526, 2013.
38. Schochl H, Grassetto A, Schlimp CJ: Management of hemorrhage in trauma. *J Cardiothorac Vasc Anesth* 27(Suppl 4):S35–S43, 2013.
39. Holcomb JB, Gumbert S: Potential value of protocols in substantially bleeding trauma patients. *Curr Opin Anaesthesiol* 26(2):215–220, 2013.
40. Darlington DN, Craig T, Gonzales MD, Schwacha MG, Cap AP, Dubick MA: Acute coagulopathy of trauma in the rat. *Shock* 39(5):440–446, 2013.
41. Cabrales P, Tsai AG, Intaglietta M: Resuscitation from hemorrhagic shock with hydroxyethyl starch and coagulation changes. *Shock* 28(4):461–467, 2007.
42. Babic AM, Josefsson EC, Bergmeier W, Wagner DD, Kaufman RM, Silberstein LE, Stossel TP, Hartwig JH, Hoffmeister KM: *In vitro* function and phagocytosis of galactosylated platelet concentrates after long-term refrigeration. *Transfusion* 47:442–451, 2007.
43. Cap AP, Spinella PC, Borgman MA, Blackbourne LH, Perkins JG: Timing and location of blood product transfusion and outcomes in massively transfused combat casualties. *J Trauma Acute Care Surg* 73(2 Suppl 1):S89–S94, 2012.

Copyright © 2014 by the Shock Society. Unauthorized reproduction of this article is prohibited.